

CLAIM AMENDMENTS

Claim 1 (currently amended). A method of generating amplified sense-oriented full-length messenger RNAs up to 4.9 kilo-base nucleotides using polymerase reactions, comprising the steps of:

(a) providing a plurality of intracellular messenger RNAs for following steps (b) to (e);

(b) contacting said messenger RNAs with a plurality of first oligodeoxythymidylate-containing primers to form a plurality of first-strand complementary DNAs, wherein said first-strand complementary DNAs are generated by reverse transcription of said messenger RNAs with extension of said first primers;

(c) permitting terminal extension of said first-strand complementary DNAs to form a plurality of polynucleotide-tailed first-strand complementary DNAs, wherein said polynucleotide-tailed first-strand complementary DNAs are tailed with multiple copies of deoxyribonucleotides;

(d) incubating denatured said polynucleotide-tailed first-strand complementary DNAs with a plurality of second RNA promoter-linked primers to form a plurality of double-stranded complementary DNAs, wherein said double-stranded complementary DNAs are generated by extension of DNA polymerase activity with said second RNA promoter-linked primers; and

(e) permitting transcription of said double-stranded complementary DNAs to form a plurality of amplified sense-oriented full-length RNAs, wherein said amplified sense-oriented full-length RNAs are generated by extension of RNA polymerase activity through the RNA promoter region of said double-stranded complementary DNAs.

Claim 2 (cancelled).

Claim 3 (original): The method as defined in claim 1, further comprising the step of cell fixation for preventing intracellular RNA degradation before the step (a).

Claims 4-6 (cancelled).

Claim 7 (original): The method as defined in claim 1, wherein said reverse transcription is an enzyme activity selected from the group consisting of AMV, M-MuLV, HIV-1 reverse transcriptase and Tth-like DNA polymerases with reverse transcription activity.

Claim 8 (previously presented): The method as defined in claim 7, wherein said enzyme activity is preformed at temperature ranged from about 55°C to about 75°C for Tth-like DNA polymerases with reverse transcription activity.

Claim 9 (previously presented): The method as defined in claim 7, wherein said first primer sequences are complementary to the tails of said messenger RNAs for the extension of reverse transcription activity in the claim 7.

Claim 10 (previously presented): The method as defined in claim 9, wherein said first primer sequences are coupled to an RNA polymerase promoter and contain about eight to about thirty copies of deoxythymidylates.

Claim 11 (previously presented): The method as defined in claim 1, wherein said denatured polynucleotide-tailed first-strand complementary DNA are formed at temperature about 94°C.

Claim 12 (previously presented): The method as defined in claim 1, wherein said DNA polymerase activity is an enzyme activity selected from the group consisting of E. coil DNA polymerase 1, Klenow fragment of E. coil DNA polymerase 1, T4 DNA polymerase, Taq DNA polymerase, Pwo DNA polymerase, Pfu DNA polymerase and Tth DNA polymerases, C. therm. Polymerase.

Claim 13 (previously presented): The method as defined in claim 12, wherein said DNA polymerase activity is achieved by C. therm. polymerases.

Claim 14 (previously presented): The method as defined in claim 12, wherein said DNA polymerase activity is performed at temperature about 70°C.

Claim 15 (original): The method as defined in claim 1, wherein said second promoter-containing primers are oligonucleotide sequences complementary to the

polynucleotide tails of said polynucleotide-tailed first-strand complementary DNAs and also coupled to an RNA polymerase promoter for transcription activity in the step (e).

Claim 16 (previously presented): The method as defined in claim 15, wherein said RNA polymerase promoter is selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase promoter.

Claim 17 (previously presented): The method as defined in claim 1, wherein said transcription is an RNA polymerase activity selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase promoter.

Claim 18 (previously presented): The method as defined in claim 17, wherein said RNA polymerase activity is preformed at temperature about 37°C.

Claim 19 (cancelled).

Claim 20 (previously presented): The method as defined in claim 1, wherein said polynucleotide-tailed first-strand complementary DNAs are tailed by terminal transferase activity.

Claim 21 (cancelled).

Claim 22 (currently amended): A method of performing improved messenger RNA amplification up to 4.9 kilo-base nucleotides, comprising the steps of:

- (a) providing a plurality of messenger RNAs for following steps (b) to (f);
- (b) generating a plurality of polynucleotide-tailed complementary DNAs from said messenger RNAs, wherein said polynucleotide-tailed complementary DNAs are reverse-transcribed from said messenger RNAs and tailed by multiple deoxynucleotides in the ends;
- (c) permitting denatured said polynucleotide-tailed complementary DNAs to form a plurality of double-stranded complementary DNAs, wherein said double-stranded complementary DNAs contain a complementary DNA sequence flanked with an RNA polymerase promoter and a polynucleotide-tail; and

(d) incubating said double-stranded complementary DNAs in a plurality of promoter- and tail-dependent extension systems, and thereby providing a plurality of amplified RNAs from said messenger RNAs.

Claim 23 (cancelled).

Claim 24 (cancelled).

Claim 25 (original): The method as defined in claim 22, wherein said messenger RNAs are protected by fixation.

Claim 26 (original): The method as defined in claim 22, wherein said messenger RNAs are protected by a plurality of RNase inhibitors.

Claims 27-28 (cancelled).

Claim 29 (original): The method as defined in claim 22, wherein said complementary DNAs are reverse-transcribed by an enzyme activity selected from the group consisting of AMV, M-MuLV, HIV-1 reverse transcriptase and Tth-like DNA polymerases with reverse transcription activity.

Claim 30 (previously presented): The method as defined in claim 22, wherein said RNA polymerase promoter is selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase promoter.

Claim 31 (previously presented): The method as defined in claim 22, wherein said polynucleotide-tailed complementary DNAs are formed by terminal transferase activity.

Claim 32 (original): The method as defined in claim 22, wherein said promoter-and-primer-dependent extension systems are a plurality of mixed polymerase activities containing RNA polymerase, DNA polymerase and reverse transcriptase.

Claim 33 (previously presented): The method as defined in claim 32, wherein said mixed polymerase activities are selected from the group consisting of T3, T7, SP6, M13 RNA polymerase and Tth DNA polymerases with reverse transcriptase activity, C. therm. polymerase.

Claim 34 (original): The method as defined in claim 22, wherein said polynucleotide-tailed complementary DNAs are tailed by terminal extension with multiple copies of same deoxynucleotides.

Claim 35 (previously presented): The method as defined in claim 34, wherein said same deoxynucleotide is selected from the group consisting of deoxyguanylate, deoxycytidylate and deoxyadenylate.

Claim 36 (cancelled).